Euphytica **129:** 281–292, 2003.

Evaluation of salt tolerance in rice genotypes by physiological characters

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Received 26 June 2001; accepted 19 July 2002

Key words: grain yield, ion selectivity, leaf area, *Oryza sativa*, salt tolerance, screening

Summary

The use of physiological characters as selection criteria in salt tolerance breeding requires the identification of the contribution each individual character makes to salt tolerance. Rice genotypes were evaluated for salt tolerance in terms of grain yield and physiological characters. Plants of twelve genotypes were grown in sand tanks in a greenhouse and irrigated with Yoshida nutrient solution. Sodium chloride and calcium chloride (5:1 molar ratio) were added at two concentrations to give moderate (4.5 dS m^{-1}) and high (8.3 dS m^{-1}) salinity treatments. One set of plants was harvested at 635 °C·d (accumulative thermal time) after planting to determine LAI and mineral ion concentrations. Another set of plants was allowed to grow to maturity. High genotypic diversity for LAI and shoot ion contents was observed. LAI contributed the most to the variation of the grain yield under salt stress. Significant correlations between LAI and yield components in both salt-tolerant and -sensitive genotypes further confirmed the significant contribution of LAI to grain yield. K-Na selectivity increased with increasing salinity. Conversely, Na-Ca selectivity decreased with increasing salinity. Significant correlations were identified between grain yield and both Na-Ca and K-Na selectivity. Highly significant ($p<0.001$) correlations were identified between Na-Ca selectivity and the rankings among genotypes for grain yield. Thus, Na-Ca selectivity could be one salt tolerance component and an useful selection criterion in screening for salt tolerance.

Abbreviations: YD – grain yield; LAI – leaf area index; DAP – days after planting

Introduction

Salinity is the most widespread and prevalent problem in irrigated agriculture. Genetic improvement for salt tolerance in major crops has become an urgent task in dealing with salinity problems in agricultural production. In traditional plant breeding for salt tolerance, overall agronomic characters such as seedling growth, survival and grain yield are usually the main selection criteria. Unfortunately, very limited success has been achieved from the previous efforts in plant breeding selecting on these characters for salt tolerance (Flowers and Yeo, 1997).

The low success in rice (*Oryza sativa* L.) salt tolerance breeding is, at least partially, due to the low selection efficiency using overall agronomic characters, lack of effective evaluation methods for salt tolerance among genotypes, and the complexity of salinity tolerance phenotypes among genotypes. Differential salinity sensitivity at various growth stages is one of the factors affecting salt tolerance phenotypes. Generally, vegetative growth in rice plants is very sensitive to salinity at young seedling stages and less sensitive at reproductive stages (Flowers & Yeo, 1981; Lutts et al., 1995). Early reproductive stages, i.e., panicle initiation (Heenan et al., 1988; Zeng et al., 2001) or pollination (Khatun & Flowers, 1995), are the most salinity-sensitive growth stages affecting the formation of yield components and grain yield. In contrast, rice is more tolerant to salinity at germination

than at other stages (Heenan et al., 1988; Khan et al., 1997). Environmental influence is another complicated factor affecting salt tolerance. Any environmental changes such as humidity and temperature will affect evapotranspiration and further influence ion transport (Shannon, 1997). This makes the evaluation of the phenotypes at field conditions very difficult.

Another approach that may be useful is to increase salinity tolerance in rice by selection of physiological characters. It has been suggested this approach may increase the efficiency in the selection procedures (Yeo & Flowers, 1986; Flowers & Yeo, 1997). Ion uptake is one such character of particular interest. Several reports in the literature indicate that Na^+ and Cl^- concentrations in shoot increase and plant growth decreases as salinity level in soils increases (Flowers & Yeo, 1981; Qadar, 1995; Shannon et al., 1998). High salinity may also affect K^+ nutrition. Increased external salinity has been shown to decrease K^+ content in shoot (Khan et al., 1992; Qadar, 1995).

Although many studies have investigated the role of Na^+ on plant growth and emphasized the importance of the mechanisms controlling Na^+ uptake, some contrasting results have been reported with respect to the interactions among ions in saline solution and their effects on plant growth and yield in rice. Muhammed and his colleagues (1987) reported that Na^+ and Cl^- concentrations in shoot decreased when Na/K or Na/Ca decreased in saline solutions. In the same study, the authors found a correlation between a decrease in the Na/K and Na/Ca ratios of the saline solution, an increase in K-Na selectivity, and an improvement of plant growth. In contrast, Yeo and Flowers (1985) reported no effect of Na/Ca in the culture solutions on the uptake of Na^+ in rice. Garcia et al. (1997) also reported a lack of evidence for K-Na selectivity in rice because the controls of Na^+ and K^+ uptake were inherited independently. This conclusion was based on a genetic study in segregating individuals for divergent sodium and potassium transport. Asch et al. (1999) found in a field study on rice cultivars irrigated with saline water that grain yield reduction was correlated with Na^+ concentration in panicles, but not with K^+ concentration in panicles at reproductive stages after booting. In another report, a highly significant correlation was found between grain yield and K^+ content in grains (Raman et al., 1986). Most recently, Asch et al. (2000b) identified a high correlation between K/Na in leaves and salinity-induced grain yield reduction at late vegetative stages in twenty rice genotypes.

The difficulty in the assessment of ion interactions and their relations with plant growth and yield may be due to possible multiple mechanisms controlling salt tolerance among genotypes. In rice, salt tolerance among genotypes may be influenced by differential Na^+ uptake pathways. There is evidence for the relation between K^+ and Na^+ (Bohra & Dörffling, 1993; Asch et al., 2000b) and for the apoplastic leakage (Yeo et al., 1987; Garcia et al., 1997). Salt tolerance among genotypes may also be influenced by discontinuous distribution of ion among or within leaves (Yeo & Flowers, 1983; Yeo & Flowers, 1986), confounding effect due to growth vigor which is dependent on different degrees of dwarf genes among genotypes (Yeo et al., 1990), and genotypic differences in controlling transpiration under salt stress (Asch et al., 1997). The determination of these salt-tolerance components among genotypes can help us model Na^+ uptake and distribution within plants (Asch et al., 1997) and eventually develop salt-tolerant cultivars by pyramiding the different tolerance components (Yeo & Flowers, 1986). Ion selectivity could be one of salt tolerance components contributing to salt tolerance in terms of grain yield, but very few studies on this topic have been reported.

The timing of sampling will be important for determining the relationships between Na^+ uptake and grain yield because some growth stages are critical for the development of yield components. Tillers per plant and spikelets per panicle were the most salinity-sensitive yield components in rice cultivar M-202 (Zeng & Shannon, 2000) and are determined at vegetative and panicle initiation stages, respectively (Hoshikawa, 1989). In a salinity-stress and -relief experiment (Zeng et al., 2001), reductions in tillers per plant only occurred when plants were stressed before panicle initiation. In the same study, reductions in spikelets per panicle were most pronounced when plants were stressed between 3-leaf stage and panicle initiation or between panicle initiation and booting stages, but the effects were minor when the plants were stressed at other stages. Asch and his colleagues (2000b) sampled rice plants a few days before panicle initiation and reported highly significant correlations between K/Na and grain yield. Therefore, the choice of growth stages just before or after panicle initiation will be appropriate for determining the relationships between physiological characters and grain yield. Furthermore, the relations between grain yield and physiological characters identified at these growth

stages can be used to predict grain yield under salt stress at relatively early growth stages.

The objectives of this study were to evaluate rice genotypes for physiological characters emphasizing ion selectivity and leaf area index at late-vegetative or early-reproductive growth stages under salinity stress, and to determine the relationships between these physiological characters and grain yield.

Materials and methods

Plant materials

Seeds of rice genotypes were received from Field Crop Research Institute at Giza of Egypt, International Rice Research Institute (IRRI) of the Philippines, and California Cooperative Rice Research Foundation Inc., at Biggs of California. These genotypes were chosen from the germplasm collections at the three sites based on their different reputation of salt tolerance in terms of agronomic performance. Among them, GZ1368-5-4 and Agami were local salt-tolerant cultivars (A.T. Badawi, Field Crop Research Institute, Giza, Egypt, personal communication, 1998) and IR63731-1-1-4-3-2 was an elite salt-tolerant breeding line (M. Akbar, International Rice Research Institute, Manila, Philippines, personal communication, 1997) based on their agronomic performance in field and greenhouse trials. Popular California cultivars M-103, M-201 and M-202 were salt-sensitive genotypes (Shannon et al., 1998; Zeng et al., 2001). This subset of germplasm included materials of both subspecies (*O. sativa* ssp. *indica* and *O. sativa* ssp. *japonica*), early to relatively late maturity, and dwarf to tall stature (Table 1).

Plant culture and salinity treatments

The experiments were conducted in the greenhouse at Riverside, California [33°58′24″ N latitude, 117°19′12″ W longitude] between March and July 1999. The plants were cultured in large tanks (122 × 61 × 46 cm deep) filled with sand (#12, Cisco Inc., CA)[1] with an average bulk density of 1.4 g cm^{-3} and irrigated with nutrient solution (Yoshida et al., 1971). The nutrient solution consisted of NH_4NO_3 (1.43 mM), $NaH_2PO_4{\cdot}2H_2O$ (0.37 mM), K_2SO4 (0.5 mM), $CaCl_2$ (1.00 mM), and $mgSO_4{\cdot}7H_2O$ (1.60 mM). Nutrient solution pH was maintained between 5.0 to 6.5 by adding sulfuric acid once a week. Irrigation solutions were prepared in 1600 L reservoirs and pumped to provide irrigation to the sand tanks. Overflow irrigation was returned to the reservoirs through drainage by gravity. Each reservoir provided irrigation to six sand tanks (replicates) three times daily for 30 min per irrigation cycle. Nutritional status of plants was monitored by periodic chemical analysis of nutrient ion concentrations in culture solutions and visual symptoms of plants for nutrient deficiency. The nutrient solutions were changed once a month to avoid possible depletion of nutrients. Seeds were planted with four rows per genotype and four genotypes per tank. The rows were spaced 7 cm apart with 15–20 seeds per row. The last row at each end of the tank was used as a border row. In addition, one plant at the ends of each row was used as a border plant. Sowing depth was less than 1 cm. Water depth was maintained 6 to 8 cm above sand surface. Twenty-five days after planting, dead plants were removed and surviving plants were thinned to 6–7 cm between plants within the rows. Air temperature ranged from 25 to 33 °C during the day and 18 to 23 °C during the night. Humidity ranged from 45 to 85%. Photosynthetically active radiation averaged 680 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ with a minimum of 100 and a maximum 1400 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ during the day. Global solar radiation maximized at 50 MJ m^{-2} d^{-1} at noon. The experiment was designed as a randomized block in a split-plot with six replicates. Salt level was the main plot factor and genotype was the sub-plot factor.

NaCl and $CaCl_2$ (5:1 molar concentration) were added to the nutrient solutions in three steps during the first week starting at the first day after planting (DAP). The stress was maintained continuously until final harvest. Electrical conductivities (EC_w) of nutrient solutions were measured with an electrical conductivity meter on alternate days. The EC_w readings of salinity treatments were averaged through the duration of salinity stress to two salt levels: 4.5 dS m^{-1} (4.1–4.8) for moderate and 8.3 dS m^{-1} (7.5–8.9) for high salinity. The non-saline control treatment, i.e., Yoshida nutrient solution only, had an EC_w of 0.90 dS m^{-1}. The final major elements in nutrient solutions were 0.37 mM Na^+, 2.0 mM Cl^-, 1 mM K^+, and 1 mM Ca^{2+} for the control, 35.7 mM Na^+, 51.7 mM Cl^-, 1 mM K^+, and 8.1 mM Ca^{2+} for the moderate salinity, and 77.5 mM Na^+, 110 mM Cl^-, 1 mM K^+, and 16.5 mM Ca^{2+} for the high salinity. Evapotranspiration (ET) demand was

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Table 1. List of rice genotypes used in the experiment

Genotype	Source	Classification of germplasm	Country of origin	Subsp	Days to[a] maturity	Height
IR63731-1-1-4-3-2	SAL119	breeding line	Philippines	Ind	140	semidwarf
Agami	HB6533	cultivar	Egypt	Jpn	125	tall
GZ1368-5-4	local	cultivar	Egypt	Ind	130	dwarf
AC26	RY96-33	anther culture derived line	Egypt	Jpn	114	semidwarf
IR50184-3B-18-2B-1	SAL013	breeding line	Philippines	Ind	150	tall
IR71657-5R-B-12PB	SST8	breeding line	Philippines	Ind	150	tall
M-103	local	cultivar	United States	Jpn	114	semidwarf
GZ178	local	cultivar	Egypt	Ind	130	semidwarf
M-201	local	cultivar	United States	Jpn	120	semidwarf
GZ177	local	cultivar	Egypt	Jpn	120	semidwarf
GZ5291-7-1-2	RY96-9	breeding line	Egypt	Jpn	114	semidwarf
M-202	local	cultivar	United States	Jpn	116	semidwarf

[a] Days to maturity were measured at Riverside, California, USA during the experiment.

determined by the decreased volume of nutrient solution during a period of 7 days before panicle initiation at 30 °C at day and 20 °C at night. The recorded ET was 4.29, 3.95 and 3.48 kg m^{-2} d^{-1} for the control, moderate and high salinity treatments, respectively. Since all genotypes were grown side by side in large tanks, the data of evapotranspiration only represented the demand averaged across all genotypes.

Plant growth measurements

Plant growth stages were measured and recorded using thermal time (°C·d, i.e., thermal degree day) (Logan & Boyland, 1983), assuming the base temperature to be 10 °C. Two plants were randomly chosen from each treatment every day when plants were approaching panicle initiation stage. The main culms were dissected under a dissecting microscope to observe the development of the young panicles. The first day that a young panicle reached 0.5 to 1.0 mM in actual length in any plant dissected for each treatment was defined as panicle initiation stage. Earliest panicle initiation occurred between 608 and 635 °C·d (44 to 46 DAP) in M-103, M-201, M-202, AC26, and GZ5291-7-1-2, and occurred between 689 and 848 °C·d (50 to 61 DAP) in the rest of genotypes under non-saline conditions. Panicle initiation was delayed by 2 to 10 days at moderate and high salt levels, respectively, in all genotypes.

Ten plants from each replicate were randomly sampled from living plants at 635 °C·d (46 DAP) in each genotype. Plants were measured individually for total leaf area per plant using LI-3100 Area Meter (LI-COR, Inc., Lincoln, Nebraska)[1]. Leaf area index (LAI) was calculated as the total leaf area per plant (not including fallen leaves) divided by its ground area in the sand tank. The same plants were washed with deionized water, dried in a forced-air oven (70 °C) and then measured for shoot dry weight, i.e., above ground biomass. Data were averaged over the ten subsamples. Dried tissues were reweighed and ground into fine powder by passing through a 60-mesh screen. Shoot concentrations of Na^+, K^+ and Ca^{2+} were determined on nitric-perchloric acid digests by inductively coupled plasma optical emission spectrometry (ICP atomic emission spectrometer, Perkin-Elmer Co., Norwalk, CT, USA)[1]. Chloride was determined on nitric-acetic acid extracts by the coulometric-amperometric titration procedure (Cotlove, 1963).

The rest of the plants were allowed to grow to maturity to determine grain yield. When seeds on the primary tillers matured (i.e., the kernels were too hard to be dented by the thumbnail), eight plants from each replicate were harvested by pulling up the plants with the roots attached. Plants were bagged individually after roots were removed. After oven-drying at 70 °C to constant weight, the seeds from the eight plants harvested were hand-threshed and weighed. Data were averaged across the eight sub-samples to determine grain weight per panicle, tillers per plant, and grain weight per plant.

Ion selectivity calculations

The principles of ion exchange theory can be used to examine Na^+ and Ca^{2+} uptake in plants (Suarez & Grieve, 1988). Based on the principles of ion exchange, the concentration of an ion in xylem is influenced by charge density at cell surface and the equivalent fractions of all the ions in xylem (Marschner, 1995). More importantly, the Na-Ca interactions at plasmalemma are believed to be related to the chemical activities of Na^+ and Ca^{2+} in the external solutions (Cramer & Läuchli, 1986). Na-Ca selectivity was calculated using Gapon selectivity constant (K_g) (Sposito, 1981):

$$K_g = (E_{na}{}^*(A_{Ca})^{0.5})/(E_{Ca}{}^*A_{Na})$$

where E represents the equivalent fraction of a given cation and A represents the activity of the ion in solution. In this way, K_g relates the equivalent fractions of the exchange ions to the activities of the ions in solution.

The K-Na selectivity was calculated using the equation described by Pitman (1976):

$$S_{k,Na} = (\text{K content}/[\text{K}]\ \text{medium}):(\text{Na content}/[\text{Na}]\ \text{medium})$$

where $S_{K,Na}$ represents K-Na selectivity; K content and Na content represent the concentrations (mmoles kg^{-1} dry wt.) of K^+ and Na^+ in shoot.

Scoring among genotypes and statistical analysis

In order to allow comparisons among genotypes, a salt-sensitive genotype, M-103, was chosen as the check, i.e., a standard against which all the other genotypes were measured. Thus, the measurements of the plants from the other genotypes in each salinity treatment were divided by the means of the check in the same salinity treatment to convert to relative values, i.e., the salt tolerance indexes. The indexes were then used for scoring and ranking among genotypes. Class intervals of indexes were artificially defined based on the ranges of the indexes for each character to allow a relatively uniform frequency of genotypes among the intervals. Scores were assigned to the class intervals from the highest to the lowest in grain yield, leaf area index, K^+ and Ca^{2+} or from the lowest to the highest in Na^+ and Cl^-.

The GLM procedure of the Statistical Analysis System (SAS Institute, 1994) was used for analysis of variance of all data. The relations between ion selectivity and salinity as well as the relations between ion selectivity and grain yield were analyzed by regressions. The best equations to fit the relations were chosen by regression procedures with selections of forward, backward and stepwise methods. The relations between grain yield and physiological characters were analyzed simultaneously by stepwise analysis. The purpose of this statistical analysis was to determine the variables significantly contributing to the variation of grain yield.

Results

Generally, the effects of salinity and genotype were highly significant ($p < 0.001$) from the analysis of variances of the characters investigated (Table 2). The interactions between salinity and genotype were also highly significant ($p < 0.001$) on most characters except Ca^{2+} (Table 2). The salt tolerance indexes varied among genotypes (Table 3). The indexes of grain yield ranged from 0.57 to 2.33 among genotypes at 8.3 dS m^{-1}. The highest indexes in LAI, i.e., 1.99–2.25, were observed in IR63731-1-1-4-3-2, Agami and GZ1368-5-4 while the lowest ones, i.e., 0.94–1.20, were observed in M-103 and GZ178. The shoot ion concentrations also varied among genotypes. The indexes of Na^+ and Cl^- ranged from 0.50 to 1.24 and from 0.70 to 1.26, respectively. The indexes of K^+ and Ca^{2+} ranged from 1.00 to 1.44 and from 0.95 to 1.27, respectively.

Genotypes were scored and ranked based on the salt tolerance indexes of grain yield at 8.3 dS m^{-1} (Table 3). The grain yield for IR63731-1-1-4-3-2 was at least two times, i.e., the indexes were greater than 2, greater than the check cultivar (M-103). This genotype was scored the highest (i.e., the smallest number of score) and ranked at the top for its salt tolerance. The grain yields for GZ5291-7-1-2 and M-202 were less than 68%, i.e., the indexes were smaller than 0.68, of that for the check cultivar. These two genotypes were scored the lowest (i.e., the largest number of score) and ranked at the bottom for their salt tolerance. Generally, the scores among genotypes on LAI were similar to those on grain yield except for M-103 and M-202. Genotypes were also scored for their shoot ion concentrations at 8.3 dS m^{-1}. In the most tolerant genotype, IR63731-1-1-4-3-2, shoot Na^+ and Cl^- concentrations were less than 59% and 77% of the check, respectively. The concentrations of K^+ and Ca^{2+} in shoots of IR63731-1-1-4-3-2 were at least 20% higher than those of the check. These results

Table 2. Mean squares and F-tests of main effects and interactions for grain yield and physiological characters under salt stress

Source[a]	df	Grain yield index	Leaf area $\times 10^{-4}$	Na^+ $\times 10^{-4}$	Cl^- $\times 10^{-4}$	K^+ $\times 10^{-3}$	Ca^{2+} $\times 10^{-3}$	K-Na selectivity	Na-Ca selectivity
Salt (S)	2	123.6***	9.0***	445***	322***	243***	59***	198***	825***
Genotype (G)	11	4.74***	0.61***	3.6***	1.8	5.2***	3.6***	6.1***	27.2***
S × G	22	1.39***	0.25***	3.5***	5.8***	1.8***	0.9	4.3***	15.2**

[a] The main effects of salt were tested using the first order interaction, replicate × salt, as the error term. The main effect of genotype and the interaction between salt and genotype were tested using the highest order interaction, replicate × salt × genotype, as the error term.
*, **, *** Significant at 0.05, 0.01 and 0.001 probability level, respectively, in *F*-tests.

Table 3. Scores among genotypes and relationships between grain yield and physiological characters at 8.3 dS m^{-1}

Genotypes	YD[a]	LAI	Ion contents				Average
			Na^+	Cl^-	K^+	Ca^{2+}	
IR63731-1-1-4-3-2	1	1	1	1	1	2	1.25
Agami	2	2	4	3	4	2	3.25
GZ1368-5-4	2	2	4	4	3	2	3.25
AC26	3	4	6	6	5	1	4.50
IR50184-3B-18-2B-1	3	2	2	2	3	4	2.75
IR71657-5R-B-12PB	4	3	2	2	2	3	2.25
M-103	4	6	5	5	6	5	5.25
GZ178	5	6	3	2	2	3	2.50
M-201	5	5	6	6	6	3	5.25
GZ177	5	4	5	4	4	5	4.50
GZ5291-7-1-2	6	5	4	4	3	4	3.75
M-202	6	3	3	3	3	6	3.75
Scores	class intervals of salt tolerance indexes[b]						
1	1.98–2.33	2.14–2.25	0.50–0.59	0.70–0.77	1.36–1.44	1.22–1.27	
2	1.63–1.97	1.99–2.13	0.60–0.70	0.78–0.83	1.23–1.35	1.17–1.21	
3	1.33–1.62	1.67–1.98	0.71–0.82	0.84–0.92	1.17–1.22	1.11–1.16	
4	0.95–1.32	1.33–1.66	0.83–0.89	0.93–0.98	1.11–1.16	1.05–1.10	
5	0.69–0.94	1.21–1.32	0.90–1.10	0.99–1.10	1.05–1.10	1.00–1.04	
6	0.57–0.68	0.94–1.20	1.11–1.24	1.11–1.26	1.00–1.04	0.95–0.99	
Regression[c] to YD							
r^2		0.422	0.089	0.088	0.068	0.004	
partial r^2		0.411	0.007	0.019	0.001	0.005	
probability		0.001	0.365	0.145	0.825	0.443	

[a] YD, grain weight per plant; LAI, leaf area index; genotypes were ranked based on the scores on grain yield in the order from the smallest number of score (the most tolerant) to the largest number of score (the least tolerant).
[b] The indexes of different characters were derived from each observation on a character divided by the mean value of the check (M-103) for that character (see Materials and methods).
[c] Regression between grain yield and different physiological characters were analyzed simultaneously using stepwise analysis: $YD = LAI \times Na^+ \times Cl^- \times K^+ \times Ca^{2+}$. The data were converted to the relative values, i.e., the salt tolerance indexes, in each character before regression analysis.

were not consistent among the other genotypes for their relations between the scores of grain yield and ion concentrations. The averages of the scores on ion contents appeared not related to the scores on grain yield among those genotypes. For example, GZ178 was ranked close to the bottom based on grain yield whereas it was scored higher than Agami based on ion concentrations. When the relationships between grain yield and different physiological characters were analyzed simultaneously (Table 3), LAI was the only character which significantly contributed to the variation of grain yield. The scores among genotypes for

Table 4. Correlation coefficients between leaf area index and yield related parameters of the most tolerant and sensitive genotypes. Regression analysis was performed using the replicates of each treatment with data combined across salt levels

	Yield related parameters		
	Grain wt per plant	Grain weight per panicle	Tillers per plant
	IR63731-1-1-4-3-2		
Leaf area index	0.26	0.1	0.22
	Agami		
Leaf area index	0.76***	0.82***	0.16
	GZ1368-5-4		
Leaf area index	0.67**	0.54*	0.01
	M-202		
Leaf area index	0.89***	0.72***	0.68**
	GZ5291-7-1-2		
Leaf area index	0.73***	0.61**	0.66**

*, **, *** Significant at 0.05 (r>0.47), 0.01 (r>0.59) and 0.001 (r>0.71) probability level, respectively, df = 16 (error).

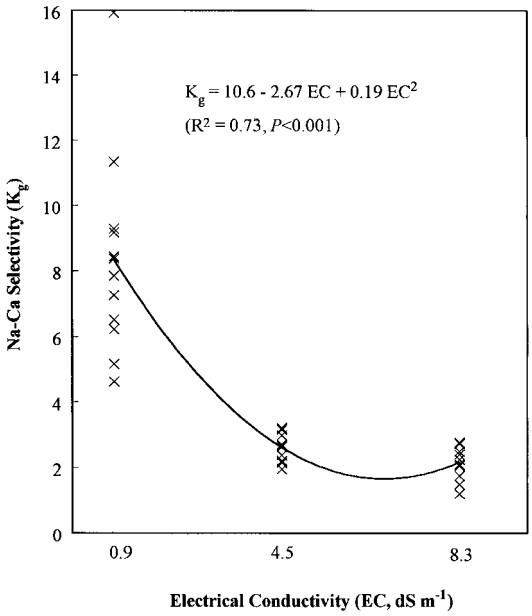

Figure 1. Regression between Na-Ca selectivity (K_g) and salinity. Regression analysis was performed on the arithmetic averages across replicates. Each point represents a genotype. A quadratic regression equation best fit the relationships between the variables.

their responses to salinity at 4.5 dS m^{-1} were similar to those at 8.3 dS m^{-1} (data not shown).

The relationships between LAI and yield components were further analyzed using the most tolerant genotypes (IR63731-4-1-1-3-2, Agami and GZ1368-5-4) and the most sensitive ones (M-202 and GZ5291-7-1-2) (Table 4). In IR63731-4-1-1-3-2, the correlations between LAI and yield components were not significant (p<0.05). However, in Agami and GZ1368-5-4, LAI was significantly (p<0.05) correlated to grain weight per panicle, although not significantly correlated to tillers per plant. In M-202 and GZ5291-7-1-2, LAI was highly significantly (p<0.01) correlated to both grain weight per panicle and tillers per plant.

The relationships between Na^+ and Ca^{2+} were analyzed using both Na/Ca and Na-Ca selectivity, i.e., Gapon selectivity constant (Kg). As illustrated in Table 5, low Na/Ca was observed in control plants of all genotypes, as expected, due to the relatively high Ca^{2+} (1 mM) and low Na^+ (0.37 mM) concentrations in control nutrient solution. Shoot Na/Ca increased with increasing salinity and the increase differed among genotypes. When salinity in nutrient solution increased from the moderate to the high salt level, the ratio did not increase in IR63731-1-1-4-3-2 but did increase to different extent in all the other genotypes. There was a preference for Na^+ over Ca^{2+} in the control plants as determined by the Na-Ca selectivity constant, K_g, (Table 5). Na-Ca selectivity was the lowest in IR63731-1-1-4-3-2 and the highest in M-202 for the control. K_g decreased with increasing salinity to different extent among genotypes. K_g remained the lowest in IR63731-1-1-4-3-2. A quadratic regression equation (R^2 = 0.73, p<0.001) based on stepwise analysis best fit the relationships between K_g and salinity (Figure 1). Note that the constant was dramatically decreased by salinity at 4.5 dS m^{-1}. Then the response became curvilinear between 4.5 and 8.3 dS m^{-1}.

The relationships between Na^+ and K^+ were analyzed using K-Na selectivity (Table 5). The data indicate a preference for K^+ over Na^+ in the control plants where the K^+ concentration (1mM) was greater than Na^+ concentration (0.37 mM) in the control-nutrient solutions. In the case of the 4.5 dS m^{-1} treatment where Na^+ increased to 35.7 mM and K^+ remained the same at 1 mM, the selectivity for K^+ over Na^+ increased by 2.9 to 5.6 times relative the values found in the control plants. When Na^+ was further increased to 77.5 mM in the 8.3 dS m^{-1} treatment, the selectivity increased among most genotypes by 0.7 to 2.1 times relative to the values measured in plants of 4.4 dS m^{-1} treatment. In the cases of AC26, GZ1368-5-4, and M-201, the selectivity was found slightly decreased compared with that of 4.5 dS m^{-1} treat-

Table 5. Salinity effect on ion ratio and selectivity in rice shoots

Genotypes	Control		4.5 dS m^{-1}		8.3 dS m^{-1}		Control		4.5 dS m^{-1}		8.3 dS m^{-1}	
	Na/Ca	K_g[a]	Na/Ca	K_g	Na/Ca	K_g	K/Na	$S_{k.Na}$[b]	K/Na	$S_{k.Na}$	K/Na	$S_{k.Na}$
IR63731-1-1-4-3-2	0.13	4.61	1.89	2.02	1.83	1.20	66.4	24.1	3.18	111.4	3.12	236.9
GZ1368-5-4	0.17	6.23	2.13	2.21	3.20	2.10	57.5	20.9	3.24	113.2	1.43	108.7
Agami	0.21	7.85	2.54	2.63	3.18	2.08	57.6	20.9	2.60	91.0	1.29	98.1
AC26	0.23	8.38	2.62	2.71	4.16	2.73	65.8	23.9	2.71	95.0	0.88	67.0
IR50184-3B-18-2B-1	0.18	6.52	2.58	2.67	2.67	1.75	55.9	20.3	2.13	74.7	2.03	154.4
IR71657-5R-B-12PB	0.14	5.16	2.08	2.15	2.26	1.48	62.5	22.7	2.86	99.9	2.28	173.3
M-103	0.25	9.18	3.06	3.16	4.23	2.77	55.8	20.3	2.09	73.2	1.11	84.0
GZ178	0.23	8.44	2.12	2.19	2.93	2.05	46.4	16.8	2.64	92.5	1.97	149.8
M-201	0.31	11.34	2.89	3.03	4.19	2.75	57.3	20.8	2.52	88.2	1.10	83.7
GZ177	0.25	9.29	2.30	3.05	3.80	2.48	57.3	20.8	2.32	81.1	1.35	102.1
GZ5291-7-1-2	0.20	7.25	3.21	3.25	3.43	2.25	66.6	24.1	1.96	68.6	1.51	114.3
M-202	0.43	15.93	3.07	3.17	3.65	2.40	41.6	15.1	2.43	84.9	1.56	118.2

[a] K_g, Na-Ca selectivity constants; [b] $S_{k.Na}$, K-Na selectivity.

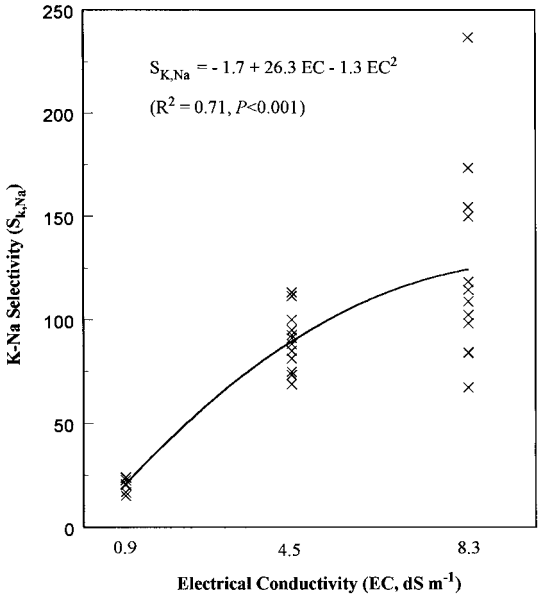

Figure 2. Regression between K-Na selectivity ($S_{K,Na}$) and salinity. Regression analysis was performed on the arithmetic averages across replicates. Each point represents a genotype. A quadratic regression equation best fit the relationships between the variables.

Table 6. Correlation coefficients between grain yield and ion selectivity. Regression analysis was performed using the replicates of each treatment with data combined across salt levels

Genotypes	YD vs K_g[a]	YD vs $S_{k.Na}$[b]
IR63731-1-1-4-3-2	0.52*	0.51*
Agami	0.48*	0.50*
GZ1368-5-4	0.63**	0.48*
AC26	0.65**	0.43
IR50184-3B-18-2B-1	0.75***	0.68**
IR71657-5R-B-12PB	0.86***	0.81***
M-103	0.84***	0.72***
GZ178	0.37	0.57*
M-201	0.53*	0.54*
GZ177	0.56*	0.63**
GZ5291-7-1-2	0.71**	0.56*
M-202	0.60**	0.84***

*, **, *** Significant at 0.05 (r>0.47), 0.01 (r>0.59) and 0.001 (r>0.71) probability level, respectively, df = 16 (error).

[a] YD, grain weight per plant; K_g, Na-Ca selectivity constants; [b] $S_{k.Na}$, K-Na selectivity.

ment. In contrast, the selectivity in IR63731-1-1-4-3-2 was 2.1 times of that at 4.5 dS m^{-1}. Again, a quadratic regression equation (R^2 = 0.71, p<0.001) based on stepwise analysis best fit the relationships between K-Na selectivity and salinity (Figure 2). Note that the selectivity dramatically increased at 4.5 dS m^{-1}. Then the response became curvilinear due to the scattered data points among genotype at 8.3 dS m^{-1}.

The relationship between ion selectivity and grain yield was analyzed by calculating the correlation coefficients between these two variables (Table 6). In most of the genotypes, the correlations were significant (p<0.05) or highly significant (p<0.01) between grain yield and both Na-Ca and K-Na selectivity. The relationship between Na-Ca selectivity and the scores

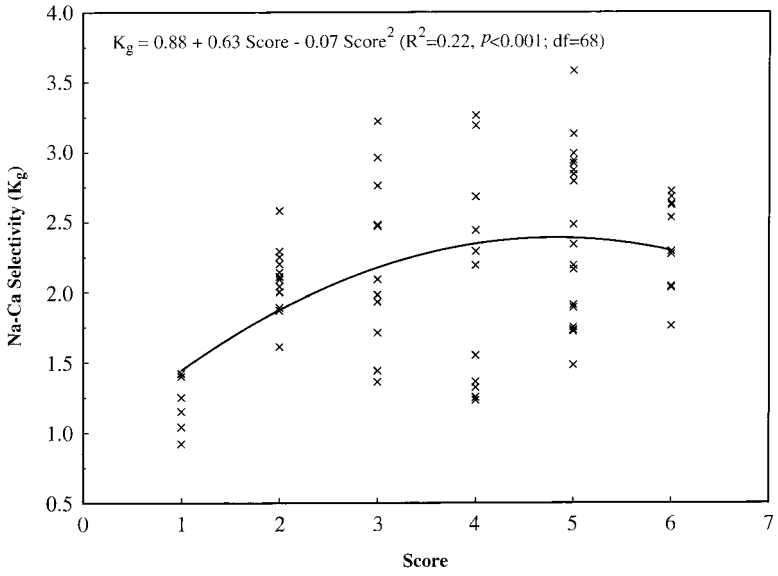

Figure 3. Relations between Na-Ca selectivity and the scores of grain yield at 8.3 dS m^{-1}. Each point represents a replicate. A quadratic equation best fit the relations between the variables.

of grain yield among genotypes at 8.3 dS m^{-1} was analyzed (Figure 3). A quadratic regression equation ($R^2 = 0.22$, $p<0.001$) based on stepwise analysis best fit the relationship. The data points for the regression between K-Na selectivity and the scores of grain yield scattered unevenly. Therefore, significant regression could not be applied to the relations.

Discussion

The highly significant correlation between grain yield and leaf area index and the similar scores among genotypes on these two characters may indicate that leaf area plays a significant role in the salt tolerance as defined by grain yield. Biomass production can be expressed as the product of the fraction of total photosynthetically active radiation (PAR) absorbed by the canopy and the efficiency of utilizing the absorbed PAR for biomass production (Monteith, 1994). The amount of PAR absorbed by a plant is strongly related to leaf area index. Wang et al. (2001) calculated accumulated PAR based on hourly inputs of predicted canopy extinction coefficients and measured leaf area index and solar radiation in salt stressed soybean. In that study, the loss of biomass production under salt stress was directly attributed to the reduction in leaf area index. In the presenting study, we have observed a highly significant effect of salinity on leaf area index and found a close relationship between LAI and grain yield. Thus, the reduction of leaf area index could be one of the major causes of yield loss under salinity stress.

The exact physiological mechanism(s) related to the loss of yield potential upon the reduction of leaf area under salt stress is unclear. Since we did not observe an increase in photosynthesis concomittant with the reductions in leaf area among the genotypes investigated (data not shown), it was reasonable to speculate that salinity-induced reductions in leaf area index at panicle initiation may result a shortage of assimilate to the developing spikelets. It has been reported that the shortage of photosynthate to reproductive organs leads to increased sterility in rice (Murty & Murty, 1981; Fukai et al., 1991). Alternatively, the reductions in plant growth or yield could be attributed to the altered assimilate partitioning such as an increase in energy consuming processes. Asch et al. (2000a) observed an increase of chlorophyll content and leaf CO_2 exchange rate at moderate salinity in three rice cultivars. The salinity-induced reductions in biomass of these cultivars could not be explained by the predicted total assimilates simulated with a crop growth model based on photosynthesis-related parameters. The authors then hypothesized that the loss of assimilates under salt stress must have occurred after their produc-

tion due to some energy-consuming processes such as osmotic adjustment and active ion transport processes.

It is possible that the correlation we observed between high grain yield per plant and LAI may not be related to some underlying physiological mechanisms but due to some morphological determinant such as the ability to maintain tillering under salt stress. In such a case, LAI would not necessarily contribute directly to grain yield. In the present study, the significant correlations between leaf area index and yield components were observed in both salt-tolerant and -sensitive genotypes. Especially in the tolerant genotypes, Agami and GZ1368-5-4, the correlations were only significant for panicle weight, but not significant for tiller number. These results confirmed the contention that LAI contributed significantly to grain yield under salt stress. It is also consistent with the hypothesis that salinity-induced reductions in leaf area index could have resulted in a shortage of assimilate to the developing spikelets. However, such a mechanism may not be universe among genotypes in rice. In the salt tolerant genotype, IR63731-1-1-4-3-2, LAI was not significantly correlated to either panicle weight or tiller number. It appears that source-sink relation in this genotype is altered under salt stress. Future work is planned to help clarify the source-sink relationships operating under salt stress and further identify the related mechanism involved in salt tolerance.

In saline soils, high salinity not only imposes osmotic and ionic stresses on plants, it also influences the uptake and transport of essential nutrients such as K^+ and Ca^{2+} (Kuiper, 1984). According to our current knowledge on ion uptake, the net ion concentration in the shoot is the result of ion uptake through selective and non-selective channels in the plant cell membrane and subsequent loading and unloading in xylem. In plants, a large number of K^+ selective membrane channels and non-selective cation channels permeable to both K^+ and Na^+ have been identified in different plant species (Amtmann & Sanders, 1999). In rice, a membrane-independent transpiration bypass flow for sodium uptake, i.e., direct apoplastic leakage into xylem in root cells, is believed to exist (Yeo et al., 1987). It has argued that this bypass flow can be a major component for Na^+ uptake in rice (Garcia et al., 1997). If most of Na^+ enter the xylem in root cells through a membrane-independent pathway, then the amount of Na^+ uptake through K^+ channels will be unmeasurable. However, this is not supported by the observations on the relationships between Na^+ and other ions. Some researchers have noticed a decrease of K^+ with the increase of Na^+ in shoots of rice plants (Qadar, 1995; Shannon et al., 1998). Our observations on the relations between Na^+ and K^+ were consistent with these earlier reports. The increase in K-Na selectivity, $S_{K,Na}$, under salt stress indicates the significance of K^+ selective channels in controlling Na^+ uptake.

It is generally thought that calcium stabilizes membrane integrity and permeability (Hanson, 1983). High concentrations of sodium reduce external calcium activity and availability at root cell membrane and increase Na^+ uptake into xylem in root cells (Reid & Smith, 2000). Calcium deficiency symptoms have been observed in rice when the molar ratios of Na^+ and Ca^{2+} in saline solutions exceeds 25 (Grieve & Fujiyama, 1987; Muhammed et al., 1987). The decreases in Na-Ca selectivity, K_g, with the increase of salinity indicate an increase in Ca^{2+} accumulation in shoots under salt stress. This phenomenon has also been observed in New Zealand spinach (*Tetragonia tetragonioides*, Pall) and red orach (*Atriplex hortensis* L.) in the presence of mixed salts in root zone (Wilson et al., 2000). In that study, the authors suggested that the plant's ability to increase Ca^{2+} accumulation with the increasing levels of external Na^+ was an adaptation by plants to salinity. The findings of the significant correlations between grain yield and ion selectivity indicate a significant contribution of ion selectivity to grain yield of rice under salt stress. It also indicates that ion selectivity may be an important factor for Na^+ uptake in plants. The highly significant ($p<0.001$) correlations between Na-Ca selectivity and the rankings among genotypes for grain yield further confirmed the importance of Na-Ca selectivity to the salt tolerance. Na-Ca Gapon constant may be an useful selection criterion in screening for salt tolerance in terms of grain yield among genotypes.

Among the genotypes tested, there was a lack of correlation between the scores on absolute ion concentrations in shoot and the scores on grain yield. Different factors may be related to this unexpected result. First, shoot ion contents were measured based on the total above ground biomass. This approach may not identify some salt tolerant components such as discontinuous ion distribution among or within leaves (Yeo & Flowers, 1986). In some genotypes such as Agami and GZ1368-5-4, tissue tolerance and ion compartmentalization may be alternative mechanisms of salt tolerance since the scores on ion contents were inconsistent with their rankings in terms of grain yield. Alternatively, the availability of Ca^{2+} to growing regions

(Grattan & Grieve, 1999) may be an important factor. Nutrient deficiency easily occurs in meristematic tissues because these regions lack vascular bundles and rely on diffusion for the nutrient supply. It is possible that the availability of Ca^{2+} to leaf and spikelet primordia contributed a significant portion to the variation of grain yield under salt stress. However, it may be that there are significant interactions among ions which make the role of each individual ion relatively minor when ions were analyzed separately.

In summary, LAI plays an important role in contributing salt tolerance as defined by grain yield although altered source-sink relations under salt stress may also be involved. Ion interactions are important in controlling Na^+ uptake in rice. Significant correlations were identified between grain yield and both Na-Ca and K-Na selectivity. Highly significant ($p<0.001$) correlations were identified between Na-Ca selectivity and the rankings among genotypes for grain yield.

Acknowledgments

This research was supported by Agricultural Technology Utilization and Transfer (ATUT) Project, no. 263-240. We thank Dr K. McKenzie (Rice Experiment Station, Biggs, CA) for providing rice seeds; Dr S. R. Grattan and Dr T.R. Kwon for critiquing the manuscript; P. Nash for the statistical analysis; and T. Donovan, J. Draper, D. Layfield, and J. Wood for their excellent technical assistance.

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